

- 76/1 -

Claims

1. An isolated culture of *Chrysosporium lucknowense* Garg 27K having accession number VKM F-3500D.

5

2. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a wild type or mutant fungus of the genus *Chrysosporium* in culture in a suitable medium, wherein the fungus is *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, or *Chrysosporium tropicum*.

10

3. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense*.

15

4. A composition having neutral and/or alkaline cellulase activity, obtained by a method which comprises growing a mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

20

5. A composition according to claim 2 wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.

6. A composition according to claim 2 wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.

25

7. A composition according to any one of claims 2-6 having optimal cellulase activity at a temperature from about 40°C to about 60°C, at a pH from about 5.0 to about 12.0.

8. A composition according to any one of claims 2-6 having at least 50% of the optimal cellulase activity, at a pH from about 6.0 to about 7.0, at a temperature from about 40°C to about 60°C.

9. A composition according to any one of claims 2-6 wherein said cellulase activity is assayed by any one of the CMCase, RBBCMCase, endovisco-metric or filter paper activity assays.

10. A substantially purified and isolated protein fraction, obtained from a composition according to claim 2 or claim 4, and having at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0 as measured by any one of the CMCase, RBBCMCase, endovisco-metric or filter paper activity assays.

11. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 25 kD and pI of about 4.

12. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 70 kD and a pI of about 4.

13. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 3.

14. An endoglucanase obtained from a fraction according to claim 10, having a molecular weight of about 43 kD and a pI of about 3.

15. A cellobiohydrolase obtained from a fraction according to claim 10, having a molecular weight of about 60 kD and a pI of about 4.

16. A substantially purified and isolated neutral and/or alkaline cellulase enzyme, isolated from a protein fraction according to claim 10, and having a pI of between about 3 and about 5.5.

5 17. A cellulase according to claim 16 wherein said cellulase possesses either endoglucanase or cellobiohydrolase activity.

10 18. A cellulase according to claim 16 wherein said cellulase retains at least 50% of its maximal cellulase activity at a pH between about 6.0 and about 7.0.

19. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 25 kD.

15 20. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 70 kD.

20 21. An endoglucanase obtained from a fraction according to claim 10 and having a molecular weight of about 43 kD.

22. A detergent composition containing one or more purified enzymes isolated from a protein fraction according to claim 10, and further comprising a surfactant.

25 23. A fabric softening composition containing one or more purified enzymes obtained from the protein fraction according to claim 10.

30 24. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus

03264153-060399

Chrysosporium, said composition having a pH between about 8.0 and about 12.0.

25. The composition of claim 24, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinsum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

26. The composition of claim 25, wherein the fungus is *Chrysosporium lucknowense*.

27. A composition according to any one of claims 24-26, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

28. A composition according to any one of claims 24-26, further comprising one or more components selected from the group consisting of pumice stones, abrasives, softeners, solvents, preservatives, bleaching agents, bluing agents, fluorescent dyes, antioxidants, solubilizers, detergents, surfactants, enzymes, builders, anti-redeposition agents, buffers, caking inhibitors, masking agents for factors inhibiting the cellulase activity, and cellulase activators.

29. The composition of claim 28, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

30. The composition of claim 24, wherein the pH is between 10.0 and about 11.0.

09284152-050399
656090-257+8260

31. The composition of claim 25, wherein the pH is between 10.0 and about 11.0.

32. The composition of claim 26, wherein the pH is between 10.0 and about 11.0.

33. The composition of claim 27, wherein the pH is between 10.0 and about 11.0.

34. The composition of claim 28, wherein the pH is between 10.0 and about 11.0.

35. The composition of claim 29, wherein the pH is between 10.0 and about 11.0.

36. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, said composition further comprising one or more components selected from the group consisting of proteinases, detergents, and surfactants.

37. The composition of claim 36, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinosum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

38. The composition of claim 37, wherein the fungus is *Chrysosporium lucknowense*.

39. A composition as described in any one of claims 36-38, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

5 40. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 124 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endovisco-metric assay, of a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

10 41. The composition of claim 40, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinsum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

15 42. The composition of claim 41, wherein the fungus is *Chrysosporium lucknowense*.

20 43. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 124 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endovisco-metric assay, of a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

25 44. The composition of claim 43, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinsum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*; and *Chrysosporium tropicum*.

30

45. The composition of claim 44, wherein the fungus is *Chrysosporium lucknowense*.

5 46. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 191 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endovisco-metric assay, of a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

10 47. The composition of claim 46, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinsum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

15 48. The composition of claim 47, wherein the fungus is *Chrysosporium lucknowense*.

20 49. A composition for the enzymatic treatment of cellulosic fibers or cellulosic fabrics, having at least 191 units of endo-1,4- β -glucanase activity per gram of dry composition, as measured by an endovisco-metric assay, of a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

25 50. The composition of claim 49, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinsum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

30

5

10

15

20

25

30

57. The composition of claim 57, wherein the fungus is *Chrysosporium lucknowense*.

58. A laundry detergent composition, comprising a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*, further comprising one or more surfactants.

59. The composition of claim 58, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinosum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

60. The composition of claim 59, wherein the fungus is *Chrysosporium lucknowense*.

61. A laundry detergent composition, comprising a cellulase isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*, further comprising one or more surfactants.

62. The composition of claim 61, wherein the fungus is selected from the group consisting of *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium pruinosum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, and *Chrysosporium tropicum*.

63. The composition of claim 62, wherein the fungus is *Chrysosporium lucknowense*.

64. A cellulase composition having cellulase activity at neutral and/or alkaline pH, obtained from a mutant or wild-type fungus of the genus *Chrysosporium*.

5 65. A cellulase composition according to claim 64, wherein the fungus is of the species *Chrysosporium lucknowense*.

66. A cellulase composition according to claim 65, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500D.

10 ~~Sub B1~~ 67. A method for producing a composition having neutral and/or alkaline cellulase activity, said method comprising growing a wild type or mutant fungus of the genus *Chrysosporium* in culture in a suitable medium.

15 68. The method, according to claim 67, wherein the fungus is *Chrysosporium lucknowense*, *Chrysosporium pannorum*, *Chrysosporium keratinophilum*, *Chrysosporium lobatum*, *Chrysosporium merdarium*, *Chrysosporium queenslandicum*, or *Chrysosporium tropicum*.

20 69. The method, according to claim 68, wherein the fungus is *Chrysosporium lucknowense*.

70. The method according to claim 69, wherein the fungus is *Chrysosporium lucknowense* Garg 27K, accession number VKM F-3500-D.

25 71. The method according to claim 67, wherein the fungus is a mutant strain of the genus *Chrysosporium*.

30 72. The method according to claim 71 wherein the fungus is a mutant strain of *Chrysosporium lucknowense* Garg 27K.

73. A method of stonewashing denim fabric or denim jeans, said method comprising treating said denim fabric or denim jeans with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

5

74. A method of biopolishing, defribillating, bleaching, dyeing, or desizing textiles comprising treating said textiles with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

75. A method of deinking or biobleaching paper or pulp, said method comprising treating said paper or pulp with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

15

76. A method for enhancing the softness or feel of cellulose or cotton-containing fabric, comprising treating said fabric with a cellulase whose amino acid sequence is encoded by a nucleic acid sequence from a wild-type or mutant fungus of the genus *Chrysosporium*.

20

77. A method according to any one of claims 73-76, wherein the cellulase is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

25

78. A method according to any one of claims 73-76, wherein the fungus is *Chrysosporium lucknowense*.

79. A method according to claim 77, wherein the fungus is *Chrysosporium lucknowense*.

30

80. A method for generating mutant strains of the genus *Chrysosporium* which produce enhanced cellulase activity at neutral and/or alkaline pH's, comprising

- (a) mutating spores of a fungus of the genus *Chrysosporium*;
- (b) culturing the spores from step (a); and
- (c) screening the cultures from step (b) for enhanced levels of neutral and/or alkaline cellulase activity.

81. The method of claim 80 wherein the mutating step comprises exposing the spores to ultraviolet light or a chemical mutagen.

82. The method of claim 81 wherein the chemical mutagen is nitrous acid, N-methyl-N'-nitro-N-nitrosoguanidine, or 4-nitroquinolone-N-oxide.

83. A mutant strain of the genus *Chrysosporium* obtained by the method of any one of claims 80-82.

84. A method of isolating genes encoding cellulase enzymes of *Chrysosporium* comprising:

- a) isolating a protein from a neutral and/or alkaline cellulase composition produced by a wild type or mutant *Chrysosporium*;
- b) sequencing all or part of the protein isolated in step (a);
- c) producing a nucleic acid probe derived from the sequence of step (b);
- d) screening a wild type or mutant *Chrysosporium* library with the nucleic acid probe of step (c);
- e) isolating a nucleic acid sequence recognized by the probe; and
- f) sequencing the nucleic acid sequence isolated in step (e).

85. A nucleic acid sequence obtained by the method of claim 84.

86. An isolated nucleic acid whose sequence encodes a cellulase enzyme, wherein the cellulase enzyme is isolated or obtained from a wild-type or mutant fungus of the genus *Chrysosporium*.

5

87. An isolated nucleic acid whose sequence encodes a cellulase according to any one of claims 11-21.

10

88. A recombinant expression vector comprising a nucleic acid sequence according to any one of claims 85-87.

15

89. A host cell containing a recombinant expression vector of claim 88.

90. A host cell according to claim 89 wherein the host cell is selected from the group consisting of yeast cells, fungal cells, plant cells, and bacterial cells.

20

91. A host cell according to claim 90 wherein the host cell is a fungal cell selected from the group consisting of *Trichoderma*, *Aspergillus*, *Humicola*, *Penicillium*, *Chrysosporium*, and *Neurospora*.

25

92. A method of culturing a fungus of the genus *Chrysosporium*, wherein the fungus is grown in a medium containing inorganic salts, carbon sources, and organic nitrogen sources, at a pH between about 5 and 8.

93. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.5 and 7.5.

94. A method of culturing a fungus of the genus *Chrysosporium* according to claim 92, wherein the pH is between about 6.9 and 7.1.